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Sinigrin degradation and *G. pallida* suppression in soil cultivated with brassicas under controlled environmental conditions

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ABSTRACT

Inter-cropping mustard and oilseed radish in potato production has been shown to suppress potato cyst nematode (PCN) reproduction in field plots. This suppressive effect is mostly attributed to the release of glucosinolate (GSL) hydrolysis products such as isothiocyanates, following chopping and incorporation of the crop biomass. This study examined the degradation of sinigrin introduced into soil where Indian mustard and oilseed radish were cultivated and the effect on PCN suppression. Glucosinolate degradation was measured by introducing sinigrin into the soil samples pre-planting, pre- and post-incorporation of the brassicas, while the soil microbial activity was quantified as the concentration of fluorescein di-acetate (FDA) g^{-1} oven dry soil. Sinigrin was significantly ($P < 0.001$) degraded pre- and post-incorporation of the brassicas cultivated in unsterilized soil. The total microbial activity increased significantly ($P < 0.001$) pre- and post-incorporation of brassicas when compared with fallow controls. Unsterilized soils planted with oilseed radish resulted in a significant ($P < 0.027$) suppression of *G. pallida* encysted eggs (30–35% mortality). Microbial activity correlated positively with *G. pallida* mortality ($R^2 = 0.94$, $P < 0.001$), but inversely with sinigrin degradation. This study has revealed that brassicas used for biofumigation may affect soil borne pests and pathogens not just after incorporation of the crop residues, but also during active growth of these plants.

1. Introduction

Plant root leachates form an important determining factor of plant–microbes interactions in the rhizosphere (Bais et al., 2006; Paterson et al., 2007; Broeckling et al., 2007). Glucosinolates (GSL) are defence-related secondary metabolites synthesised by members of the Brassicaceae (Fahey et al., 2001) including oilseed radish and mustard. The hydrolysis of GSL catalysed by the enzyme myrosinase is characterised by the release of nitriles, epithionitriles, oxazolidine-2-thiones, thiocyanates and isothiocyanates depending on the side chain of the GSL and the reaction conditions (Bennett et al., 2004; Grubb and Abel, 2006). This system can influence and alter the microbial community in the rhizosphere of Brassicaceae (Rumberger and Marschner, 2003). Stimulation of ectomychorhizal growth by root exudates of Brassicaceae have been reported (Zeng et al., 2003). By contrast, root exudation of benzyl isothiocyanate (ITC) by garlic mustard (Rodgers et al., 2008) has been reported to inhibit ectomycorrhizal fungal growth, affecting the ability of native trees to compete for nutrients (Wolfe et al., 2008) and thus altering the functioning of the ecosystem (Rodgers et al., 2008). The inability of Brassicaceae plant roots to form arbuscular mycorrhizal has also been implicated with the toxic products released from GSL hydrolysis (Vierheilig et al., 2000; Roberts and Anderson, 2001). Suppressive effects on field populations of *Globodera pallida* by developing mustard and oilseed radish plants cultivated as an inter-crop in potato production has recently been reported (Ngala et al., 2014). The mechanisms underlying this suppressive effect are not fully understood.

As demonstrated by McCully et al. (2008), brassica crops do release GSL into the soil during cell replenishment. However, intact GSL occurring on their own are understood to be non-toxic until they become hydrolysed in the presence of the enzyme myrosinase. Roots of Brassicaceae plants such as oilseed rape (Choesin and Boerner, 1991) and mustard (Schreiner and Koide, 1993) are also known to release GSL directly into the rhizosphere which directly affects root colonising microbes. The exudation of ITC from actively growing roots into the soil has been reported (Elliott and Stowe, 1971) possibly due to superficial cell damage during growth and development of the roots. The release of myrosinase into the soil by developing brassica root tissues is unknown. However, reports

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of myrosinase producing soil microbes are well documented. Borek et al. (1996) demonstrated myrosinase activity in soil extracts from a field cultivated with rapeseed but soil extracts from pasture showed little/no activity. These authors also specified that myrosinase activity was four fold greater in soil sampled directly from rapeseed rows than activity observed in the soil sampled in-between the rapeseed rows. Sakorn et al. (1999) reported both high myrosinase activity and sinigrin degradation when a strain of *Aspergillus* sp. was incubated in a medium containing sinigrin. Linking these previous findings to field observations of PCN suppression in Brassicaceae cultivated field experiments (Ngala et al., 2014), two hypotheses were formulated; (i) root tissues may be exudate GSL from alongside endogenous myrosinase for hydrolysis, or (ii) exuded GSL could be hydrolysed by extracellular myrosinase released by soil microbes (Borek et al., 1996).

The experiments reported were undertaken to investigate the underlying cause of PCN suppression observed in brassica cultivated field plots (partial biofumigation) as reported in Ngala et al. (2014). The objectives were to: (i) test the partial biofumigation effect of selected biofumigant brassicas on *G. pallida* under glasshouse controlled conditions; (ii) monitor the hydrolysis of 2-propanyl GSL (sinigrin) in soil sampled during the growth and development of selected brassicas under glasshouse controlled conditions and (iii) monitor soil microbial activity following brassica plant development and after chopping and incorporation.

2. Materials and methods

2.1. Experimental set-up and treatments

Two glasshouse experiments (GE) were conducted during 2013/2014 to monitor the underlying cause of the PCN suppression in the field experiments (Ngala et al., 2014). The first experiment (GE-1) had five treatments (Table 1) each of which was replicated five times. In a follow-up experiment (GE-2), an additional treatment was introduced which included sterilised fallow soil. The soil used for the experiments was sandy loam (71.7% sand, 18.5% silt, 9.8% clay, pH 6.28) (MAFF, 1986), and was samples within the top 30 cm from a previous field experimental site (UK ordinance survey grid reference: SJ 70386 21266, Shropshire, UK) lying on a gentle slope. The soil was taken to the glasshouse where it was homogenised, coarsely passed via a sieve with pore sizes of 1 cm^2 and used to three-quarter fill 1.7 l pots. Soil sterilisation was achieved via autoclaving for 1.5 h (Camplex Plant-care Soil Steriliser, 50 Hz, 3000 Watts, Themoforce Ltd., Cumbria, England), before cooling overnight prior to potting. During the setting up of pots, 50 cysts of *G. pallida* (each of size ≥500 μm in diameter) wrapped in a 250 μm nylon mesh (cyst sachets), were placed at a depth of 10 cm in each pot. The pots were randomized and blocked in the direction of light source in the glasshouse.

Raphanus sativus (oilseed radish) and *Brassica juncea* (Indian mustard) were sown into their designated pots at the supplier's recommended seed rates (Table 1) resulting in 17 plants per pot for each species. Seedlings were therefore thinned to 17 plants per pot at the two leave stage (two weeks post-germination). The experiment was maintained in the glasshouse at a day/night temperature of 15/5 °C respectively with a 16 h photoperiod. Application of water to each pot was done twice weekly for the first three weeks post-germination by applying 200 ml to each pot and thereafter, the pots were maintained at 26 ± 6% volumetric water content (vwc) with the aid of a moisture meter (Theta Prop Type HH2, Delta-T Devices Ltd., Burwell, UK).

At ten weeks post planting, the cyst sachets were removed for assessments and the brassicaceous tissue was chopped (2–5 cm in length) using a plant shredder (Viking GE150 Shredder, Tom's Garden Equipment, Ashburton, UK) and homogenised with the soil before re-potting. The setup was left for six weeks while the soil moisture was maintained at 20 ± 3% vwc before the final soil samples were collected to measure GSL degradation and microbial activity post-incorporation.

Table 1
Treatments used in the glasshouse experiments. Glasshouse Experiment-1 had 5 treatments with 5 replicates and Glasshouse Experiment-2 had an additional treatment (Sterilized Fallow soil).

Treatments	Variety	Sterilization	Seed rates	*Cysts pot^{-1}
Glasshouse Experiment-1				
Fallow	N/A	No	N/A	50
Brassica juncea	Caliente 99	No	8 kg ha^{-1}	50
B. juncea	Caliente 99	Yes	8 kg ha^{-1}	50
Raphanus sativus	Bento	No	20 kg ha^{-1}	50
R. sativus	Bento	Yes	20 kg ha^{-1}	50
Glasshouse Experiment-2				
Fallow	N/A	No	N/A	50
Fallow	N/A	Yes	N/A	50
B. juncea	Caliente 99	No	8 kg ha^{-1}	50
B. juncea	Caliente 99	Yes	8 kg ha^{-1}	50
R. sativus	Bento	No	20 kg ha^{-1}	50
R. sativus	Bento	Yes	20 kg ha^{-1}	50

N/A = not applicable, * = *G. pallida*.

2.2. Hydrolysis of glucosinolates in soil

Soil samples were collected from the experimental pots at the following times to determine the hydrolysis of sinigrin (2-propenyl GSL) and microbial activity:

- Pre-planting of brassicas,
- Pre-incorporation of brassicas and
- Six weeks post-incorporation of brassicas.

At each time of sampling, the soil from each pot was homogenised and sub-samples of 50 g were placed into sterile polythene bags and taken to the laboratory where they were either immediately processed or stored below 4 °C prior to processing. Each soil sample was passed through a 1 mm sieve and completely homogenised before a 2.5 g sub-sample was placed into a 15 ml polypropylene tube and spiked with a 1 ml aliquot of 5 mM sinigrin solution. The tubes were securely capped and incubated in darkness at room temperature for 96 h before the sinigrin was re-extracted with 70% Methanol at room temperature following the method described by Gimsing et al. (2005). The extract was ion-exchange column purified and desulfated before being subjected to HPLC analysis as described in Ngala et al. (2014) to determine the degradation of sinigrin in the individual treatments.

2.3. Total microbial enzyme activity analysis via fluorescein di-acetate hydrolysis

In a bid to measure the presence/concentration of GSL degrading soil microbes, fluorescein di-acetate (FDA) hydrolysis assay was conducted to measure total microbial enzyme activity. In this assay, the production of a bright yellow colouration (the FDA product, fluorescein) indicates microbial activity, and the intensity as determine by a spectrophotometer indicates the level of activity. Therefore, following soil sieving as described above, sub-samples were processed according to the protocol outlined in Solaiman (2007). The colour intensity was quantified using a spectrophotometer at a *UV* absorbance of 490 nm and results were expressed as μg fluorescein produced g^{-1} dry soil. This analysis was conducted for GE-2.